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Erysiphe russellii in Korea supported by new morphological and sequence analyses

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Abstract—Morphological and rDNA ITS sequence analyses were conducted on three strains obtained from leaf lesions on creeping wood sorrel (*Oxalis corniculata*) covered by a powdery mildew. No chasmothecia were observed, but the symptoms and traits of conidiophores and conidia agree well with those of *Erysiphe russellii* (previously recorded from Korea as *Microsphaera russellii*). Phylogenetic analysis shows that the three strains share 97.9-98.06 % identity with *Erysiphe pisi* (FJ378867) but form a separate *E. russellii* clade with a sequence of that species based on Japanese material. This supports the occurrence of *Erysiphe russellii* in Korea.

Key words—*Erysiphales,* pathogen, phylogeny, taxonomy

Introduction

Species in the genus *Erysiphe* (*Ascomycota*, *Erysiphales*, *Erysiphaceae*) are plant pathogens that cause powdery mildews (Takamatsu et al. 2015). The *Erysiphaceae* currently comprises about 873 species belonging to 16 genera (Braun & Cook 2012). It includes five tribes and two basal genera, which have been described by Mori et al. (2000) and Takamatsu et al. (2005). Creeping wood sorrel (*Oxalis corniculata, Oxalidaceae*) is a generally low-growing, spreading, perennial weedy or invasive plant with a yellow flower and rounded trifoliate clover-like leaves. It is widely distributed worldwide and occurs in diverse habitats, including arable fields and gardens or wastelands, but its origin is unknown. The leaves of the creeping wood sorrel are sometimes used as edible plant; it has a tangy taste due to a rich amount

Table 1. *Erysiphe* samples analyzed.

Species	Sample no.	Source (host)	Locality	GenBank ITS no.
E. diffusa	SMK15414	*Glycine soja*	South Korea	AB078812
	MUMH1162	*Glycine soja*	Japan	AB078813
	MUMH791	*Glycine max*	Japan	AB078800
	MUMH1463	*Glycine max*	USA	AB078810
	MUMH1451	*Glycine max*	Vietnam	AB078809
	MUMH56	*Amphicarpaea edgeworthii* var. *japonica*	NI	AB015934
E. glycines	MUMH52	*Desmodium oxyphyllum*	NI	AB015927
E. howeana	NI	*Oenothera biennis*	USA	AF011301
E. palczewskii	PMWI	*Caragana arborescens*	USA	GQ497277
	PMQB	*Caragana arborescens*	Canada	GQ497276
E. peruviana	DAG08-36	*Tecoma capensis*	USA	GU117107
	08-3618S	*Tecoma capensis*	USA	GU987124
E. pisi	FF 06	Pea	USA	FJ378867
	GH 07	Pea	USA	FJ378872
	GH 04	Pea	USA	FJ378870
	Lathyrus 07	Pea	USA	FJ378879
	GE 07	Pea	USA	FJ378869
	NI	*Lathyrus latifolius*	USA	AF011306
E. polygoni	bean 2A	*Phaseolus vulgaris*	Mexico	GU570595
	DNA14	*Rumex obtusifolius*	Japan	LC009892
	MUMH2431	*Polygonum* sp.	Argentina	LC010013
	UC1512295	*Polygonum arenastrum*	USA	AF011307
	UC1512308	*Rumex crispus*	USA	AF011308
E. sp. [1]	EB 2004	*Phaseolus vulgaris*	Brazil	AY739109
	DB 121211	*Oenothera biennis*	Italy	JQ288740
	3D	*Senna septentrionalis*	Mexico	JQ730709
E. sp. **[2]**	**EML-COR2**	***Oxalis corniculata***	**Korea**	**KP941751**
	EML-COR3	***Oxalis corniculata***	**Korea**	**KP941752**
	MUMH0105	***Oxalis corniculata***	**Japan**	**LC009922**
E. trifoliorum	NI	*Trifolium repens*	China	JQ282732
	VPRI 22166	NI	NI	AF298542
	MUMH29s	*Trifolium vulgaris*	NI	AB015913

NI: Not identified.

Two unknown species are designated by [1] (still unknown) and [2] (supported as *E. russellii* after complete analysis).

of vitamin C and is traditionally used for drinks by infusing the leaves in hot water, sweetening, and then chilling.

Between July and October 2010 and in August 2012, symptoms of powdery mildew were observed on creeping wood sorrel plants in Suwon, Gwangju and Cheongyang in Korea. *Erysiphe russellii* (Clinton) U. Braun & S. Takam. has been reported from several species of *Oxalis* worldwide (Farr & Rossman 2015) and is the only powdery mildew that has been previously reported on creeping wood sorrel in Korea, based on morphology of the asexual morph. Therefore, the morphology of the new Korean powdery mildew collections on *Oxalis corniculata* has been re-examined, supplemented by a molecular phylogenetic analysis of rDNA ITS sequences, to confirm the identity of the causal agent of the powdery mildew as *E. russellii*. Molecular analyses of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) is a well-established method for fungal identifications at species level (White et al. 1990), which have been frequently applied to powdery mildews (Takamatsu et al. 1999, Cunnington et al. 2003).

Materials & methods

Sampling and morphological observations

Fresh samples infected by the powdery mildew pathogen were collected and examined by stereo and light microscopy. Voucher specimens were deposited in EML (Environmental Microbiology Laboratory) Fungarium (EMLF; Chonnam National University, Gwangju, Korea as EML-COR1 (obtained from a garden located in Suwon in July 2010), EML-COR2 (obtained from a pot for red pepper in Gwangju in August 2010), and EML-COR3 (obtained from a greenhouse located in Cheongyang in late October 2010).

DNA extraction, polymerase chain reaction amplification, and sequencing

Total genomic DNA was isolated from 50–100 mg of powdery mildew conidia and mycelia using the Accuprep® Genomic DNA Extraction Kit (Bioneer Corp., Daejeon, Korea). The rDNA ITS region was amplified using primers ITS1F and LR5F following Lee (2012a). The 20 µL PCR amplification mixture contained 2 µL DNA template, 1.5 µL per primer (5 pM/µL), 1 µL Accupower® PCR premix (containing Taq DNA polymerase, dNTPs, buffer, and tracking dye; Bioneer Corp.), and 14 µL of sterile water. Amplification parameters were an initial denaturation step at 95°C for 5 min, 30 cycles of denaturation at 94°C for 1 min + annealing at 54°C for 30 s + extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were purified using the Accuprep® PCR Purification Kit (Bioneer Corp.). DNA sequencing was performed in an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc.).

Phylogenetic analyses

After initial sequence alignment using CLUSTAL_X (Thompson et al. 1997), the alignment was edited manually. Sequence analyses were performed using MEGA 6 (Tamura et al. 2013) with the default settings. Phylogenetic trees were generated from the data using maximum-likelihood (ML), neighbor joining (NJ), and maximum parsimony (MP) methods. The Kimura 2-parameter model was selected as the best model to construct trees for NJ. Nearest-neighbor- interchange (NNI) was selected for the ML heuristic method, and the initial tree for ML was set automatically. The MP analysis was conducted with the heuristic search option using the tree-bisection-reconstruction (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting tree was tested with a bootstrap (BS) analysis using 1000 replications in the ML, NJ, and MP analyses. Tree scores, including tree length, consistency index (CI), and retention index (RI), were also calculated. *Erysiphe glycines* was used as outgroup. Sequences from this study and those from GenBank database are listed in Table 1.

Taxonomy

Erysiphe russellii (Clinton) U. Braun & S. Takam., Schlechtendalia 4: 13 (2000) Fig. 1

≡ *Microsphaera russellii* Clinton, in Peck, Rep. (Annual) New York Stat. Mus. 26: 80 (1874).

= *Oidium oxalidis* McAlpine, Proc. Roy. Soc. Victoria, N.S., 6: 219 (1894).

Mycelium on leaves, effuse, covering the entire leaf surface, white, thin, powdery. Hyphae somewhat straight to slightly wavy, septate, branched. Appressoria lobed and single or in opposite pairs. Conidiophores on top of hyphal mother cells, erect, 76.6–134 × 4.7–6.8 µm, with lecythiform to clavate apices above two or three cells. Conidia formed singly; primary conidia ellipsoidal to fusiform; secondary conidia, slender, cylindrical to oblong with slightly obtuse or truncate apices, 24–55 × 13.5–22 µm. Chasmothecia not observed.

Specimens examined—**KOREA**, Cheongyang, 36°27'45"N 126°51'34"E (EML-COR3), Gwangju, 35°10'0"N 126°55'0"E (EML-COR2), Suwon, 37°17'27"N 127°00'32"E (EML-COR1), on *Oxalis corniculata* L., October 2010, H.B. Lee. Voucher specimens (KOSPFG0000134896) were deposited in the herbarium of the National Institute of Biological Resources (NIBR), Incheon, Korea.

Discussion

In Korea, *Erysiphe russellii* was previously reported by Shin (2000, as *Microsphaera russellii* based on conidial morphology) as the causal pathogen on creeping wood sorrel. Previous studies (Takamatsu et al. 2003, 2015; Lee 2012a,b, 2013a,b, 2015; Lee et al. 2013) have successfully

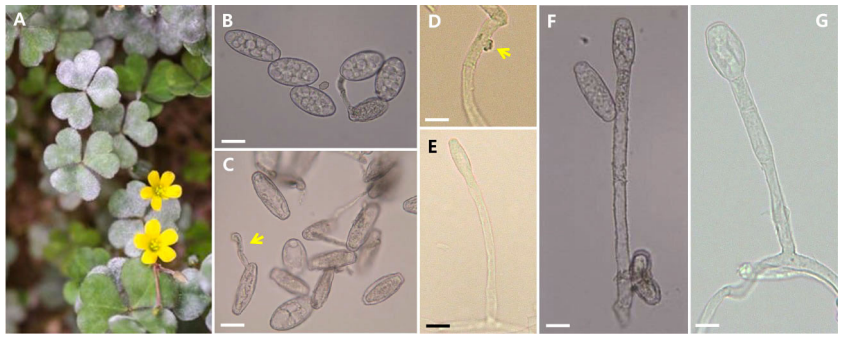

Fig. 1 Powdery mildew symptoms and the morphology of the causal pathogen, *Erysiphe* sp. EML-COR3 isolate. A. Powdery mildew symptoms on the leaves of creeping wood sorrel; B & C. Conidia and conidia with germ tube (yellow arrow); D. Appressoria on hyphae (yellow arrow). E-G. Conidiophores. Bars: B, D, F, G =20 µm; C, E=50 µm.

used rDNA ITS sequence data to identify *Erysiphe* species. Therefore, we conducted phylogenetic analysis based on three Korean strains and a single strain from Japan (LC009922) to confirm the identity of the Korean collections. ITS sequences from the three Korean strains were compared to those from related species retrieved from GenBank. Our sequence analysis (Fig. 2) clustered the sequences from strains EML-COR1, EML-COR2, and EML-COR3 together with a Japanese sequence in a separate clade in the phylogenetic tree assignable to *Erysiphe russellii*, supporting it as a distinct species. The morphology of the asexual morph most closely resembled that of *E. russellii* described by Braun (1987) and Shin (2000).

In the phylogenetic analyses, two small clades, highlighted by question marks emerged which could not be satisfactorily named (Fig. 2).

Various additional powdery mildew species have been reported on species of *Oxalis* worldwide, e.g. *Golovinomyces orontii* (Castagne) Heluta (= *Erysiphe polyphaga* Hammarl.), *Leveillula oxalidicola* T.Z. Liu & U. Braun, and a species of *Phyllactinia* (Braun & Cook 2012, Farr & Rossman 2015). This report is the first to verify the presence of *E. russellii* on creeping wood sorrel in Korea by DNA sequence comparisons. The pathogenicity of the biotrophic fungus was confirmed through inoculation experiments made by gently pressing infected leaves onto mature leaves of healthy creeping wood sorrel plants in a glasshouse. Seven days after inoculation, symptoms similar to those observed under natural infection were developed on the inoculated leaves of creeping wood sorrel plants (data not shown). More studies on

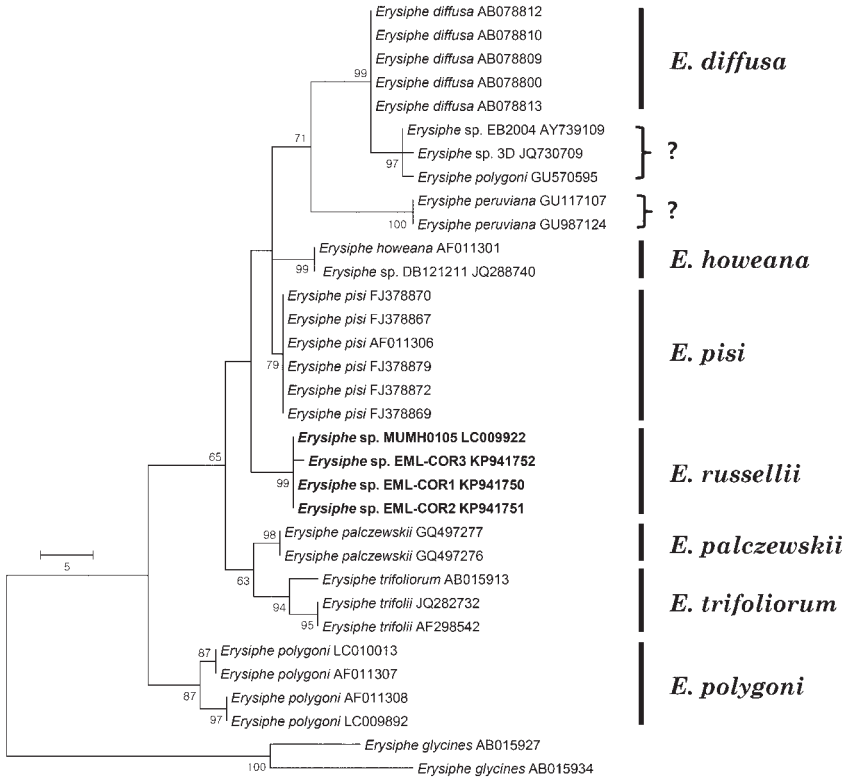

Fig. 2 Phylogenetic status of the EML-COR1, EML-COR2, and EML-COR3 and MUMH0105 isolates and related species based on maximum parsimony (MP) of the internal transcribed spacer (ITS) region sequences. Numbers at the nodes indicate the bootstrap values (>50 %) from 1000 replications. *Erysiphe glycines* was selected as outgroup. The thick line is based on a previous phylogenetic system constructed by Takamatsu et al. (2015). Two small clades, highlighted by question marks, emerged that could not be satisfactorily named. The scale bar indicates the number of nucleotide substitutions per site.

the distribution of powdery mildews on *Oxalis* in Korea, including a search for the sexual morph, are necessary. Moreover, sequence data based on North America samples (USA is the type locality of *E. russellii*) and material from Australia (type locality of *Oidium oxalidis*) are urgently required for comparison.

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Literature cited

Braun U. 1987. A monograph of the *Erysiphales* (powdery mildews). Beiheft zur Nova Hedwigia 89: 1–700.

Braun U, Cook RTA. 2012. Taxonomic manual of the *Erysiphales* (powdery mildews). CBS Biodiversity Series 11: 707 p.

Cunnington JH, Takamatsu S, Lawrie AC, Pascoe IG. 2003. Molecular identification of anamorphic powdery mildews (*Erysiphales*). Australasian Plant Pathology 32: 421–428. http://dx.doi.org/10.1071/AP03045

Farr DF, Rossman AY. 2015. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved from http://nt.ars-grin.gov/fungaldatabases/

Lee HB. 2012a. First report of powdery mildew caused by *Erysiphe arcuata* on lanceleaf coreopsis (*Coreopsis lanceolata*) in Korea. Plant Disease 96: 1827 p. http://dx.doi.org/10.1094/PDIS-08-12-0754-PDN

Lee HB. 2012b. Molecular phylogenetic status of Korean strain of *Podosphaera xanthii*, a causal pathogen of powdery mildew on Japanese thistle (*Cirsium japonicum*) in Korea. Journal of Microbiol 6: 1075–1080. http://dx.doi.org/10.1007/s12275-012-2618-z

Lee HB. 2013a. First report of *Oidium* anamorph of *Erysiphe hypophylla* causing powdery mildew on leafy lespedeza (*Lespedeza cyrtobotrya*) in Korea. Plant Disease 97: 287 p. http://dx.doi.org/10.1094/PDIS-08-12-0774-PDN

Lee HB. 2013b. First report of powdery mildew caused by *Erysiphe heraclei* on curled dock (*Rumex crispus*) in South Korea. Plant Disease 97: 427 p. http://dx.doi.org/10.1094/PDIS-10-12-0904-PDN

Lee HB. 2015. First report of powdery mildew caused by *Erysiphe alphitoides* on *Quercus acutissima* on Korea. Plant Disease 99: 889 p. http://dx.doi.org/10.1094/PDIS-08-14-0848-PDN

Lee HB, Kim CJ, Mun HY. 2013. First report of powdery mildew on Spanish needles (*Bidens bipinnata*) caused by *Podosphaera xanthii* in Korea. Plant Disease 97: 1385 p. http://dx.doi.org/10.1094/PDIS-10-12-0966-PDN

Mori Y, Sato Y, Takamatsu S. 2000. Evolutionary analysis of the powdery mildew fungi using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92: 74–93. http://dx.doi.org/10.2307/3761452

Shin HD. 2000. *Erysiphaceae* of Korea. Plant pathogens of Korea 1. NIAST. Suwon. Korea.

Tamura K, Stecher G, Peterson D, Filioski A, Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology Evolution 30: 2725–2729. http://dx.doi.org/10.1093/molbev/mst197

Takamatsu S, Tetsuya H, Yukio S, Yukihiko N. 1999. Phylogenetic relationships of *Microsphaera* and *Erysiphe* section *Erysiphe* (powdery mildews) inferred from the rDNA ITS sequences. Mycoscience 40: 259–268. http://dx.doi.org/10.1007/BF02463963

Takamatsu S, Sato Y, Mimuro SK. 2003. *Erysiphe wadae*: a new species of *Erysiphe* sect. *Uncinula* on Japanese beech. Mycoscience 44: 165–171. http://dx.doi.org/10.1007/s10267003-0100-9

Takamatsu S, Braun U, Limkaisang S. 2005. Phylogenetic relationships and generic affinity of *Uncinula septata* inferred from nuclear rDNA sequences. Mycoscience 46: 9–16. http://dx.doi.org/10.1007/s10267-004-0205-9

Takamatsu S, Ito H, Shiroya Y, Kiss L, Heluta V. 2015. First comprehensive phylogenetic analysis of the genus *Erysiphe* (*Erysiphales, Erysiphaceae*) I. The microsphaera lineage. Mycologia 107: 475–489. http://dx.doi.org/10.3852/15-007

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUST_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. http://dx.doi.org/10.1093/nar/25.24.4876

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics. 315–322 in Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR Protocols: A guide to methods and applications, Academic Press, San Diego. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1